PHYLOGENETIC POSITION AND TAXONOMIC CLASSIFICATION OF *AETHIONEMA TRINERVIUM* (BRASSICACEAE): A MORPHOLOGICALLY VARIABLE SUBSHRUB FROM SOUTHWESTERN ASIA[1]

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Abstract

Due in part to its distinctive and variable morphology, traditional taxonomy has not resolved the systematic position of *Aethionema trinervium* Boiss., which has been previously placed in several different genera including *Aethionema* R. Br., *Eunomia* DC., *Hutchinsia* R. Br., and *Thlaspi* L. Using sequence data from the ITS-1 and ITS-2 regions of ribosomal DNA, *A. trinervium* has been added to a previously published data set composed of proposed congeners and related taxa in order to clarify its phylogenetic relationships. Our molecular analysis indicates that *A. trinervium* is not a member of *Aethionema*, but is instead highly nested within *Vania* F. K. Mey., a segregate lineage of the nonmonophyletic *Thlaspi* s.l. Based on these results and subsequent taxonomic recommendations, we transfer *A. trinervium* to *Vania*, described from Iran, as the new combination *V. trinervia* (DC.) Khosravi, Jacquemoud, Menke, Mumm. & Mohsenz. Furthermore, we have lectotypified the species.

Key words: *Aethionema*, Brassicaceae, ITS sequence analysis, *Noccaea*, phylogenetic relationships, Southwest Asia, *Thlaspi*, *Vania*.

Returning to previously analyzed molecular data sets with additional data is a valid and efficient method for increasing our overall phylogenetic knowledge and for giving additional emphasis to more narrowly focused questions, such as clarifying the taxonomic placement of a single problematic species (Mummenhoff et al., 1997a, b, 2001; Crawford et al., 2001; Warwick et al., 2006a).

In the present study, we demonstrate the utility of sequences from the ITS region of nuclear ribosomal DNA to resolve the generic affinities of a problematic species in the Brassicaceae, i.e., *Aethionema trinervium* Boiss., a perennial subshrub. It is distributed in Afghanistan, Turkmenistan, Iran, Transcaucasia, Turkey, and northern Iraq (Hedge, 1965, 1968). The plants are essentially glabrous and woody at the base, with the ascending stems remaining herbaceous (Hedge, 1965). Leaves are strongly palmately veined, amplexicaul, mostly 10–20 mm in length, and variously shaped from lanceolate to ovate to oblong with the basal auricles varying from almost absent to sagittately elongate. Leaf shape may vary dramatically on a single individual between the proximal and distal leaves of the same shoot. The inflorescence is racemose, starting as a condensed head but elongating in fruit (Hedge, 1965, 1968). Sepals are erect, petals are white and 1-nerved at the base, filaments are free, and anthers are apiculate (Hedge, 1965). Silicles are angustiseptate and 4–10 mm in length, and they vary from lanceolate to oblong. Furthermore, fruit valve wings may be present or absent. If present, the wings vary in shape from broadly obcordate or squarely

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oblong or reduced to a narrow margin (Hedge, 1965, 1968). The seed surface is smooth (Hedge, 1965). Individuals collected at higher elevations (above 3000 m) tend to have markedly smaller leaves and display a subpulvinate habit (Hedge, 1965, 1968).

Morphologically, the evidence for placing the species in *Aethionema* R. Br. is equivocal, with many vegetative features of *A. trinervium* being atypical for the genus. However, there are examples in which some unusual morphological states are approached in at least one other species of *Aethionema*. For example, the prominent palmate leaf venation in the annual *A. arabicum* (L.) Andrz. ex DC. resembles that of *A. trinervium*. Also, while no other *Aethionema* species possesses auriculate or amplexicaul leaves, the cordate and short petiolate leaves of *A. cordatum* Boiss. approach such a condition. Fructal morphology is quite variable within *Aethionema*, as it is throughout much of Brassicaceae, but all core taxa within *Aethionema* possess fruit with membranously winged valves. As stated before, the presence of wings is a variable trait in *A. trinervium*, which has been a principal reason for classification problems.

Taxonomists who did not accept that *Aethionema trinervium* belongs to the genus *Aethionema* placed the species in a number of other genera. Originally described as *Hutchinsia trinervia* DC. by de Candolle (1821), it was also later placed in the genera *Iberidella* Boiss. (Boissier, 1841), *Aethionema* DC. (Boissier, 1867), *Eunomia* DC. (Prantl, 1891), and *Thlaspi* L. (Mozaffarian, 1996). *Eunomia trinervia* (DC.) Prantl was recombined on the basis of the presence of median nectary glands. Schulz (1933) also later recognized *E. trinervia* in his own familial classification. This conclusion is not easy to follow because in *A. trinervium*, as in *A. salmasium* Boiss., median nectary glands are absent. However, the lateral nectaries are somewhat connected, thus possibly giving the impression of median glands. Based on some morphological and cytological characters, Mozaffarian (1996) included *A. trinervium* in *Thlaspi* as *T. trinervium* (DC.) Mozaff., and this species was also treated as *T. trinervium* in the recent species checklist of the Brassicaceae (Warwick et al., 2006b). Apparently, Mozaffarian did not recognize the main phylogenetic lineages within *Thlaspi* s.l. suggested by Meyer (1973, 1979, 1991), which are based on the analysis of seed coat anatomy and later generally supported by molecular studies (Mummenhoff & Koch, 1994; Mummenhoff et al., 1997a, b; Koch et al., 2001). Thus, it is unclear to which *Thlaspi* s.l. segregate *A. trinervium* should be assigned. Recent broadly sampled molecular studies of Brassicaceae (Khosravi, 2001; Koch et al., 2001; Beilstein et al., 2006) also proved that *Thlaspi* s.l. is a polyphyletic taxon and that the members of *Thlaspi* s. str. are distantly related to the other *Thlaspi* s.l. lineages. In the most recent systematic treatment of the family, Al-Shehbaz et al. (2006) placed members of *Thlaspi* s. str., along with other genera (e.g., *Alliaria* Heist. ex Fabr., *Pachyphragma* Rchb., *Peltaria* Jacq.), in tribe Thlaspideae and remaining *Thlaspi* s.l. segregates in the tribe Noccaeeae. In summing up its taxonomic history, it is clear that *A. trinervium* has long been a poorly understood species for which previous taxonomic classification attempts have proven both vague and unstable.

Furthermore, the morphology within *Aethionema trinervium* is exceptionally variable, and some of this variation appears to show geographic structure (Davis et al., 1965). Herbarium specimens may suggest a gradual reduction in fruit valve wing size and an increase in leaf auricle size as the species ranges from west to east. In addition, four separately described taxa are often either reduced to synonymy with *A. trinervium* or treated as a variety thereof. Two of the synonymous names, *Iberidella ovalifolia* Boiss. and *I. sagittata* Boiss. (later *A. sagittatum* (Boiss.) Boiss.), were described by Boissier in 1842. They apparently differ from each other in the relative prominence of the leaf auricles. Such variation has been treated within the scope of *A. trinervium* in some floras (Hedge, 1965, 1968; Hedge & Lamond, 1980), although others distinguish between *A. trinervium* and *A. sagittatum* (Shishkin & Vasilchenko, 1948; Karjagin, 1953; Avetisian, 1966).

Furthermore, Boissier (1867) separated *Aethionema salmasium* from *A. trinervium* on the basis of fruit wing differences. However, *A. salmasium* was not accepted by Hedge (1965, 1968), apparently due to overlapping patterns of morphological variation. However, other national floras do recognize both species as distinct (Karjagin, 1953; Avetisian, 1966; Khintibidze, 1979).

Perhaps most different from typical *Aethionema trinervium* is *A. apterocarpum* Rech. f. & Aellen, which is described from material in the Iranian provinces of North Khorasan, South Khorasan, and Razavi Khorasan, where the taxon is endemic (Hedge, 1968). It was reclassified as *A. trinervium* var. *apterocarpum* (Rech. f. & Aellen) Hedge by Hedge (1968). It is characterized by wingless fruit valves that are further distinguished by the fruit shape itself being shorter and more ovate than in typical *A. trinervium*. Also, the inflorescence peduncle usually elongates earlier in floral development than typical *A. trinervium*, with each flower's pedicel separated by approximately a centimeter of peduncle length at the time of anthesis. In contrast, typical *A. trinervium* inflorescences remain as compacted racemes at anthesis but elongate later during fruit development. The biogeographic and genetic relationships between the morphological variants of *A. trinervium* remain unclear. The problem is introduced here, but clear answers await completion of a more comprehensive population-level genetic analysis of what may potentially be a multispecies complex. Thus, the goal of this present study is to use sequence variation of the nuclear ITS of ribosomal DNA (ITS-1 and ITS-2) of *A. trinervium* and representatives of putative related genera, especially *Aethionema* and *Thlaspi* s.l. segregates, advocated generic rank by Meyer (1973, 1979, 1991), to clarify the phylogenetic position of *A. trinervium*.

MATERIALS AND METHODS

SAMPLING

In the current study, we have included representatives of *Aethionema* and *Thlaspi* s.l. along with other taxa previously shown to be closely related to *Thlaspi* s.l. (Zunk et al., 1996; Mummenhoff et al., 1997a, b; Koch & Mummenhoff, 2001). Because *Aethionema* is sister to all remaining Brassicaceae (Zunk et al., 1996, 1999; Galloway et al., 1998; Koch et al., 2001, 2003) and *A. trinervium* might be nested in *Aethionema*, *Cleome lutea* Hook., a member of the Cleomaceae (sensu Hall et al., 2002), the sister family of the Brassicaceae, was selected as the outgroup. The final data set comprised 39 taxa. ITS sequences were generated for *A. trinervium*. Other sequences were obtained from GenBank (Table 1).

DNA EXTRACTION, AMPLIFICATION, AND SEQUENCING

Total DNA was isolated from dried leaf material of *Aethionema trinervium* (IRAN. **Kerman Province:** Ravar, Sood Kuh, 5 Sep. 2004, *Mirtadzadini 24985*, voucher in Herbarium of Shiraz University) using a modified CTAB method (Aras et al., 2003). Double-stranded DNA of the complete ITS region, including the 5.8S ribosomal DNA (rDNA) gene, was amplified by 35 cycles of symmetric polymerase chain reaction (PCR) using ITS primers initially designed by White et al. (1990) and modified by Mummenhoff et al. (1997a). The PCR profile was a hot start with 5 min. at 94°C, and 35 cycles of amplification (1 min. 94°C, 1 min. 50°C, 1 min. 72°C), with final elongation for 10 min. at 72°C. PCR products were purified using the High Pure PCR Product Purification Kit (Roche Diagnostics–Applied Science, Mannheim, Germany). Sequencing reactions were run on an ABI 377XL automated sequencer (Applied Biosystems, Weiterstadt, Germany).

DATA ANALYSIS

DNA sequences were aligned with those obtained from GenBank using Clustal W version 1.8 (Thompson et al., 1994). Multiple alignment parameters were set to 12 for gap opening penalty and 0.1 for gap extensions penalty. Alignments were confirmed manually using sequential pairwise comparisons. Regions with ambiguous alignment were eliminated from phylogenetic analyses. The alignment is available from the first author. Maximum parsimony analyses of the aligned sequences of the ITS data set were conducted using the computer program PAUP*, version 4.0b10 (Swofford, 2000). The most parsimonious trees were generated using the heuristic search method, with tree bisection-reconnection (TBR) branch swapping, equal-weighted characters, 1000 random additions of the sampled taxa, and 100 trees saved per replicate. Sets of equally most parsimonious trees were summarized by a strict consensus tree. Bootstrap tests (Felsenstein, 1985) were performed using 500 replicates with heuristic search settings identical to those of the original search. Pairwise distance sequence divergence of ITS was calculated for each accession pair in PAUP* using the Kimura two-parameter model (Kimura, 1980).

RESULTS

The aligned data matrix contained 403 positions after removing regions with alignment ambiguities. Of these, 188 (46.6%) were potentially phylogenetically informative, 126 (31.3%) were invariant, and 89 sites (22.1%) were autapomorphic. The heuristic search resulted in 126 most parsimonious trees of 718 steps with a consistency index of 0.5615. The results of the phylogenetic analysis are shown in Figure 1. Two major clades can be recognized on the strict consensus tree (Fig. 1). The first lineage (referred to here as *Thlaspi* clade) comprises all the species traditionally subsumed under *Thlaspi* s.l. along with other genera recently shown to be closely related to *Thlaspi* s.l. (Mummenhoff & Koch, 1994; Mummenhoff et al., 1997a, b; Koch & Mummenhoff, 2001; Koch & Al-Shehbaz, 2004). Most representatives of this lineage belong to tribes Noccaeeae and Thlaspideae. Also, *Aethionema trinervium* clearly belongs here, and it is found in a strongly supported clade (100% bootstrap support) along with *Vania campylophylla* F. K. Mey. and *V. kurdica* (Hedge) F. K. Mey. in the tribe Noccaeeae. The second lineage represents the genus *Aethionema* and is referred to here as the *Aethionema* clade (tribe Aethionemeae). The genetic divergence between these two main clades is

Table 1. List of taxa used for the current study.

Taxon	Source	GenBank accession number
Aethionema arabicum (L.) Andrz. ex DC.	Hong et al., 2003	AY254539
A. elongatum Boiss.	Koch et al., unpublished	DQ518386
A. grandiflorum Boiss. & Hohen.	Bailey et al., 2006	DQ452067
A. saxatile (L.) R. Br.	Mummenhoff et al., 2005	AJ862697
A. trinervium Boiss.	Present study	FM180111
Alliaria petiolata (M. Bieb.) Cavara & Grande	Mummenhoff et al., 2005	AJ862703/AJ862704
Cleome lutea Hook.	O'Kane & Al-Shehbaz, 2003	AF137588
Cochlearia aucheri Boiss.	Koch & Mummenhoff, 2001	AF336202/AF336203
C. megalosperma (Maire) R. Vogt	Koch & Mummenhoff, 2001	AF336208/AF336209
C. sempervivum Boiss. & Balansa	Peer et al., 2003	AY261529
C. sintenisii Hausskn. ex Bornm.	Koch & Mummenhoff, 2001	AF336204/AF336205
Peltaria turkmena Lipsky	Koch & Mummenhoff, 2001	AF336212/AF336213
Teesdalia nudicaulis (L.) R. Br.	Koch & Mummenhoff, 2001	AF336214/AF336215
Thlaspi alliaceum L.	Koch & Mummenhoff, 2001	AF336156/AF336157
T. alpinum Crantz	Koch & Al-Shehbaz, 2004	AY154812
T. arvense L.	Koch & Mummenhoff, 2001	AF336152/AF336153
T. bulbosum Boiss.	Koch & Mummenhoff, 2001	AF336200/AF336201
T. caerulescens J. Presl & C. Presl	Koch & Mummenhoff, 2001	AF336188/AF336189
T. calaminare Lej. & Courtois	Koch & Mummenhoff, 2001	AF336192/AF336193
T. cepaeifolium (Wulfen) W. D. J. Koch	Koch & Al-Shehbaz, 2004	AF336198/AF336199
T. ceratocarpum N. Busch	Koch & Mummenhoff, 2001	AF336154/AF336155
T. cilicicum (Schott & Kotschy) Hayek	Koch & Mummenhoff, 2001	AF336166/AF336167
T. crassiusculum (F. K. Mey.) Greuter & Burdet	Koch & Mummenhoff, 2001	AF336206/AF336207
T. densiflorum Boiss. & Kotschy	Koch & Al-Shehbaz, 2004	AY154816
T. elegans Boiss.	Koch & Mummenhoff, 2001	AF336160/AF336161
T. goesingense Halácsy	Peer et al., 2003	AY261528
T. granatense Boiss. & Reut.	Koch & Mummenhoff, 2001	AF336176/AF336177
T. hastulatum Steven ex DC.	Koch & Mummenhoff, 2001	AF336164/AF336165
T. jankae A. Kern.	Koch & Al-Shehbaz, 2004	AY154817
T. lilacinum Boiss. & A. Huet	Koch & Mummenhoff, 2001	AF336162/AF336163
T. macranthum N. Busch	Koch & Mummenhoff, 2001	AF336194 /AF336195
T. montanum L.	Koch & Mummenhoff, 2001	AF336196/AF336197
T. natolicum Boiss.	Koch & Mummenhoff, 2001	AF336184/AF336185
T. orbiculatum Steven ex DC.	Koch & Mummenhoff, 2001	AF336172/AF336173
T. oxyceras (Boiss.) Hedge	Koch & Mummenhoff, 2001	AF336158/AF336159
T. perfoliatum L.	Koch & Mummenhoff, 2001	AF336180/AF336181
T. szowitsianum Boiss.	Koch & Mummenhoff, 2001	AF336174/AF336175
T. umbellatum Steven ex DC.	Koch & Mummenhoff, 2001	AF336186/AF336187
Vania campylophylla F. K. Mey.	Koch & Mummenhoff, 2001	AF336168/AF336169
V. kurdica (Hedge) F. K. Mey.	Koch & Mummenhoff, 2001	AF336170/AF336171

considerably high. ITS sequence divergence between *A. trinervium* and other *Aethionema* species ranges between 34.5% (*A. trinervium* vs. *A. elongatum* Boiss.) and 39.5% (*A. trinervium* vs. *A. grandiflorum* Boiss. & Hohen.). On the other hand, the sequence divergence between *A. trinervium* and *Thlaspi* s.l. species is significantly lower and ranges between 2% (*A. trinervium* vs. *V. campylophylla*) and 21.5% (*A. trinervium* vs. *T. hastulatum* (Steven ex DC.) Hedge). This is additional evidence that *A. trinervium* is more closely related to *Thlaspi* s.l. taxa than to *Aethionema*.

Discussion

In the current study, we used evidence from nuclear ITS sequences to clarify the generic status and phylogenetic relationships of *Aethionema trinervium*, a species variously assigned to different genera in different traditional classifications (e.g., *Hutchinsia* R. Br., de Candolle, 1821; *Iberidella*, Boissier, 1841; *Aethionema*, Boissier, 1867; *Eunomia*, Prantl, 1891; *Thlaspi* s.l., Mozaffarian, 1996). These genera are all taxonomically critical, but for a better understanding

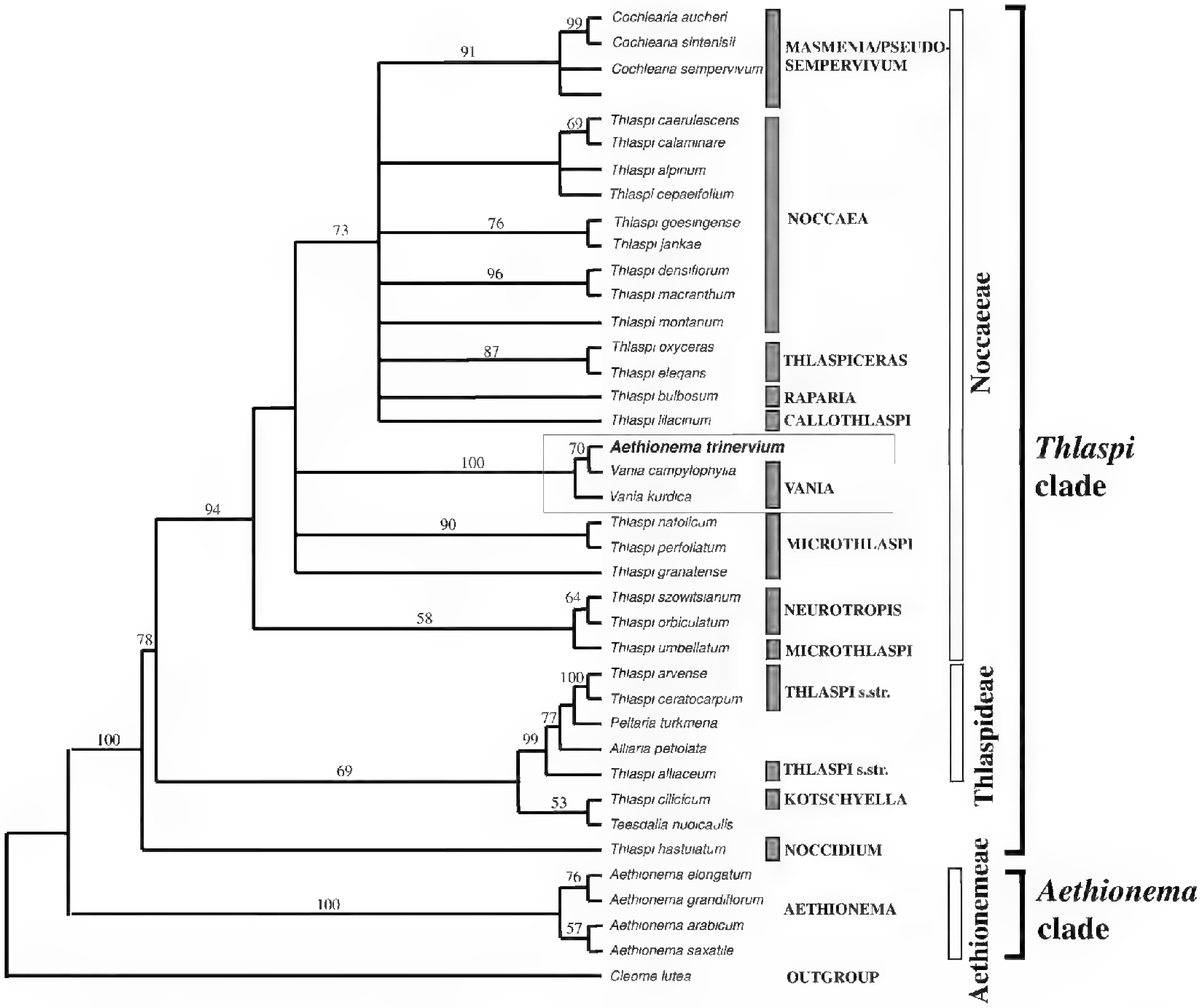

Figure 1. Strict consensus tree of 126 most parsimonious Fitch trees (tree length — 718 steps). Bootstrap values more than 50% are indicated above branches. Generic grouping by Meyer (1973, 1979, 1991) is indicated by grey vertical bars. *Alliaria petiolata* (M. Bieb.) Cavara & Grande and *Peltaria turkmena* Lipsky were not recognized by Meyer as *Thlaspi* s. str. (Koch et al., 2001; Mummenhoff et al., 2001). Tribal grouping by Al-Shehbaz et al. (2006) is indicated by white bars.

of the phylogenetic discussion, these taxa need some closer inspection.

Meyer (1973, 1979) divided *Thlaspi* s.l. into 12 segregate genera based primarily on differences in seed sculpture and seed-coat anatomy in contrast to morphological fruit characters prone to homoplasy and used in traditional treatments (see Meyer, 1991; Mummenhoff et al., 1997b; Koch & Mummenhoff, 2001; Meyer et al., 2001a). The status of these segregates varied from complete rejection (Greuter et al., 1986) to partial acceptance (Al-Shehbaz, 2002) to complete acceptance (Czerepanov, 1995). *Thlaspi* s.l. has been subjected to extensive molecular studies to test the validity of Meyer's (1973, 1979, 1991, 2001a, b) segregates. Recently, Koch and Mummenhoff (2001) summarized a decade of phylogenetic studies on *Thlaspi* and pointed out that, with the exception of certain segregates (e.g., *Microthlaspi* F. K. Mey.), most of Meyer's segregates represent monophyletic groups supported by molecular data. Furthermore, species previously placed in *Cochlearia* L. sect. *Pseudosempervivum* Boiss. (e.g., *C. sempervivum* Boiss. & Balansa, *C. aucheri* Boiss., *C. sintenisii* Hausskn. ex Bornm.) are nested within *Thlaspi* s.l. and are better treated either as *Masmenia* F. K. Mey. or *Pseudosempervivum* (Boiss.) Grossh. (Fig. 1). Finally, *Thlaspi* s. str. is more closely related to *Peltaria*, *Alliaria*, and *Thlaspi* s.l. segregates *Noccidium* F. K. Mey. and *Kotschyella* F. K. Mey. than to any other of Meyer's segregates of *Thlaspi* s.l. (Fig. 1). Except for *Thlaspi* s. str., *Noccidium*, and *Kotschyella*, the other *Thlaspi* s.l. segregates of Meyer (1973, 1979) group with *Noccaea* Moench in a well-supported clade. It was suggested by Al-Shehbaz et al. (2006) that only a few of Meyer's segregates might deserve recognition (Fig. 1); these include *Thlaspi* s. str., *Neurotropis* (DC.) F. K. Mey., and only part of *Microthlaspi*, and the remaining segregates should perhaps best be treated as synonyms of *Noccaea* (Al-Shehbaz et al., 2006). However, our data also indicate that *Vania* F. K. Mey. might

Table 2. Distribution of morphological and cytological data in *Aethionema*, *A. trinervium*, *Thlaspi* s. str., *Vania*, and *Noccaea*.

Character	*Aethionema*	*A. trinervium*	*Thlaspi* s. str.	*Vania*	*Noccaea*
Habit	annual or perennial	perennial	annual	perennial	perennial
Basal leaves	not rosulate	subrosulate	not rosulate	subrosulate	rosulate or subrosulate
Basal leaf shape	sessile, linear or oblong	oblong or subulate	spatulate or oblanceolate	oblong or subulate	spatulate
Stem leaf shape	linear, oblong, or ovate	oblong, ovate, or linear-lanceolate	oblong or lanceolate	oblong, ovate, or linear-lanceolate	broad ovate, ovate, or oblong
Base of stem leaf blades	very rarely auriculate	exauriculate or + auriculate	auriculate	exauriculate or + auriculate	auriculate or exauriculate
Leaf attachment to stem	very rarely amplexicaul	amplexicaul	amplexicaul or sagittate	amplexicaul	amplexicaul or sagittate
Sepals	+ bisaccate	not saccate	not saccate	not saccate	not saccate
No. of veins on petal claws	3	1	1	1	1
Anthers	apiculate or not apiculate	apiculate	not apiculate	apiculate	not apiculate
Shape of lateral nectaries	semiglobose	crescent	crescent	crescent	crescent
Seed surface	smooth or minutely papillose	smooth	striate or reticulate	smooth	smooth
Seed mucilage	present or absent	absent	absent	absent	absent or slightly present
Base chromosome number	mostly x — 11, 12	x — 7	x — 7	x — 7	x — 7

deserve recognition because this lineage is well separated from the core *Noccaea* group consisting of *Masmenia*/*Pseudosempervivum*, *Noccaea* s. str., *Thlaspiceras* F. K. Mey., *Raparia* F. K. Mey., and *Callothlaspi* F. K. Mey. (Fig. 1). In the most recent systematic treatment of the family, Al-Shehbaz et al. (2006) placed the members of *Thlaspi* s. str. in tribe Thlaspideae and the remaining *Thlaspi* s.l. lineages in tribe Noccaeeae. The primary difference between the Thlaspideae and Noccaeeae is the presence of striate or coarsely reticulate seeds and often palmately veined basal leaves in the former tribe.

The genus *Aethionema* comprises approximately 60 species and shows tremendous variation in habit (annual herbs to shrubs), floral structure (filaments with or without appendages), floral color, fruit morphology, and base chromosome numbers (n — 7, 8, 11, 12, 14, 16, 18, 21, 22, 24, 30; Appel & Al-Shehbaz, 2003). *Aethionema* was long thought to be closely related to *Thlaspi* s.l. (Schulz, 1936; Al-Shehbaz, 1986), but molecular data clearly demonstrate that *Aethionema* is distantly related to *Thlaspi* s.l. and instead is sister to the rest of the Brassicaceae (Koch et al., 2003; Al-Shehbaz et al., 2006, and references therein). Khosravi (1989) recognized two unrelated groups of *Aethionema* species, one group with one nerve on the petal's claw, half moon–like lateral nectar glands, and a base chromosome number of x — 7. These taxa were previously assigned to *Eunomia* (e.g., *Aethionema iberidium* (Boiss.) Boiss. — *Eunomia iberidea* Boiss., *A. oppositifolium* Boiss. — *E. oppositifolia* DC., *A. rotundifolium* Boiss. — *E. rotundifolia* C. A. Mey., *A. caespitosum* (Boiss.) Boiss. — *E. caespitosa* (Boiss.) O. E. Schulz, and *A. trinervium* (DC.) Boiss. — *E. trinervia* (DC.) Prantl). The second group, the *Aethionema* core group (including remaining *Aethionema* species and *Moriera* Boiss.), is characterized by three nerves on the claw, semiglobose lateral nectaries, and a base chromosome number of x — 11, 12 (Table 2). This group belongs to tribe Aethionemeae. An in-depth phylogenetic analysis of the tribe Aethionemeae is currently being investigated by one of the authors (M.M.).

The genus *Eunomia* has been most recently treated as a synonym of *Aethionema* (Appel & Al-Shehbaz, 2003) or *Iberis* L. (Hall et al., 2002), but the latter phylogenetic analysis demonstrated that *I. oppositifolia* Pers. (— *Eunomia oppositifolia*) is neither related to *Aethionema grandiflorum* nor *I. amara* L. Therefore, it remains unclear if *Eunomia* should be recognized as an independent genus. Recent *ndhF* and *trnL-F* data (Khosravi, unpublished data; Menke, unpublished data) provided evidence that *E. oppositifolia* is a close relative of *Noccaea*. Summing up, the systematic position of *Eunomia* needs to be resolved, as does the taxonomic placement of the ca. 16 species previously assigned to it.

Our molecular study clearly shows that *Aethionema trinervium* is outside the *Aethionema* clade and is instead well nested in the *Thlaspi* clade (Fig. 1). *Aethionema trinervium* is placed with 100% bootstrap support into a lineage along with *Vania kurdica* and *V. campylophylla*. *Vania* is one of the 12 segregate genera defined by Meyer (1991). Members of the *Vania* lineage are xeromorphic, cushion-forming plants growing at altitudes between 3000 and 4000 m. Detailed morphological examination of *A. trinervium* demonstrates that it is very similar to the typical representatives of the *Vania* lineage (for a description, see Meyer, 2006). *Vania* species have a pulvinate or subpulvinate habit and simple glabrous stems. The basal leaves are spatulate, entire, and show a firm texture, while the stem leaves are amplexicaul or sessile, oblong or oblong-lanceolate, the flowers have white petals and apiculate anthers, the fruits are oblong or obcordate, wingless or almost wingless, and the seeds are smooth and not mucilaginous. The base chromosome number is x – 7. Meyer (1973) described three species of *Vania*: *V. campylophylla*, *V. kurdica*, and *V. pulvinata* F. K. Mey. All *Vania* species and *A. trinervium* have a connective tooth at the top of the anther never seen in *Noccaea*.

Nomenclature and Typification

Morphological and molecular data strongly support the transfer of *Aethionema trinervium* to *Vania*, and we propose the following new combination:

Vania trinervia (DC.) Khosravi, Jacquemoud, Menke, Mumm. & Mohsenz., comb. nov. Basionym: *Hutchinsia trinervia* DC., Reg. Veg. Syst. Nat. 2: 387. 1821. TYPE: [Iran. Alwand Kuh:] "Hab. in Persiae monte Elwind. Michaux. Olivier," *herb. Olivier 1822* (lectotype, designated here, G-DC!). Figure 2.

There is a handwritten label on the lectotype, with the following detail: "*Hutchinsia trinervia* [scripsit A.-P. DC.] / *Mont evlind* [sic, scripsit Olivier?] / *herb. Olivier 1822* [scripsit x, non A.-P. DC.]." The lectotype is the plant specimen attached to that label, on the right side of the sheet (see Fig. 2, barcode G00131229). It should be noted that the "1822" annotation represents the year of accession by de Candolle, not a date of gathering. Additionally, there is an isolectotype, a single sheet and single specimen, at Paris (P 00633350 photo!) with the following handwritten label: "*Hutchinsia trinervia* DC. [scripsit A.-P. DC.] / *Iberidella trinervia* Boiss. / *Aethionema*—*Bss. Fl. Or.* [scripsit x] / *Amadan. / Mont Elvind. / Olivier et Bruguière* [scripsit y]." An annotation label by A.-P. de Candolle, "*Hutchinsia trinervia* DC.," also appears on the isolectotype sheet.

Three syntypes were also known to us for consideration. The first specimen (G-DC!, 1: 178, n.6; barcode G 00131230, one sheet with one specimen) carries a handwritten label: "*Iberis / Perse* [scripsit x]." A small envelope containing a dissected flower is attached to this sheet and is annotated by de Candolle "*petala aequalia / an Lepia?*" This was part of the original collection by Michaux, and this syntype is mounted on the same physical sheet as our designated lectotype, on the left part (see Fig. 2). The second candidate syntype at P (P 006633349, P photo!, one sheet with two specimens) bears a handwritten label: "*Hutchinsia trinervia DC.* [scripsit A.-P. DC.] / *Iberidella trinervia Boiss. / Aethionem*—*Bss. Fl. Or.* [scripsit x] / *Perse. Michaux* [scripsit y]." Finally, a third candidate syntype was examined (G!, ex hb. Delessert, one sheet with one specimen), which is represented by plate 2, tab. 53, in Delessert's *Icones selectae plantarum* (Delessert, 1824: 16). This third syntype has the handwritten label: "*Hutchinsia trinervia DC.* [scripsit x] / *Aethionema trinervium Boiss. / J. Briquet 1912* [scripsit Briquet] / *Michaux (Herb. De Perse)* [scripsit y]." The G syntype has an annotation label glued on the first, which states "*Thlaspi / trinervatum* [scripsit ?] / *Hutchinsia trinervia* DC. [scripsit A.-P. DC.]."

There is no doubt that Michaux was the first botanist who collected *Hutchinsia trinervia*. However, the taxon was already considered morphologically variable by Boissier (1842: 188–189), who regarded it as a representative of the genus *Aethionema* in his treatment of Cruciferae (Boissier, 1867: 341–343). Therefore, because further taxonomic research may perhaps lead to division of *A. trinervium* s.l. into different taxa, the exact geographic origin of the lectotype needs special attention. Thus, preference was given to the collections by Olivier and Bruguière where the label indications clearly fit the protologue locality (de Candolle, 1821: 387). Moreover, the Olivier and Brugière specimens not only bear flowers, but also young fruits. Although immature, these fruits enabled de Candolle to resolve the unsatisfactory floral dissection made on a Michaux collection ("an Lepia?" written on the envelope, see above) and to describe a new species of the genus *Hutchinsia*. Further, the iconograph of *Hutchinsia trinervia* by the French illustrator J. F. Turpin was one of the many (ca. 500) engravings published in *Icones selectae plantarum* (Delessert, 1824: 16, tab. 53) (Fig. 3) with descriptions by de Candolle.

Nevertheless, we attempted to find more information about the Persian travels of Michaux, as well as of his

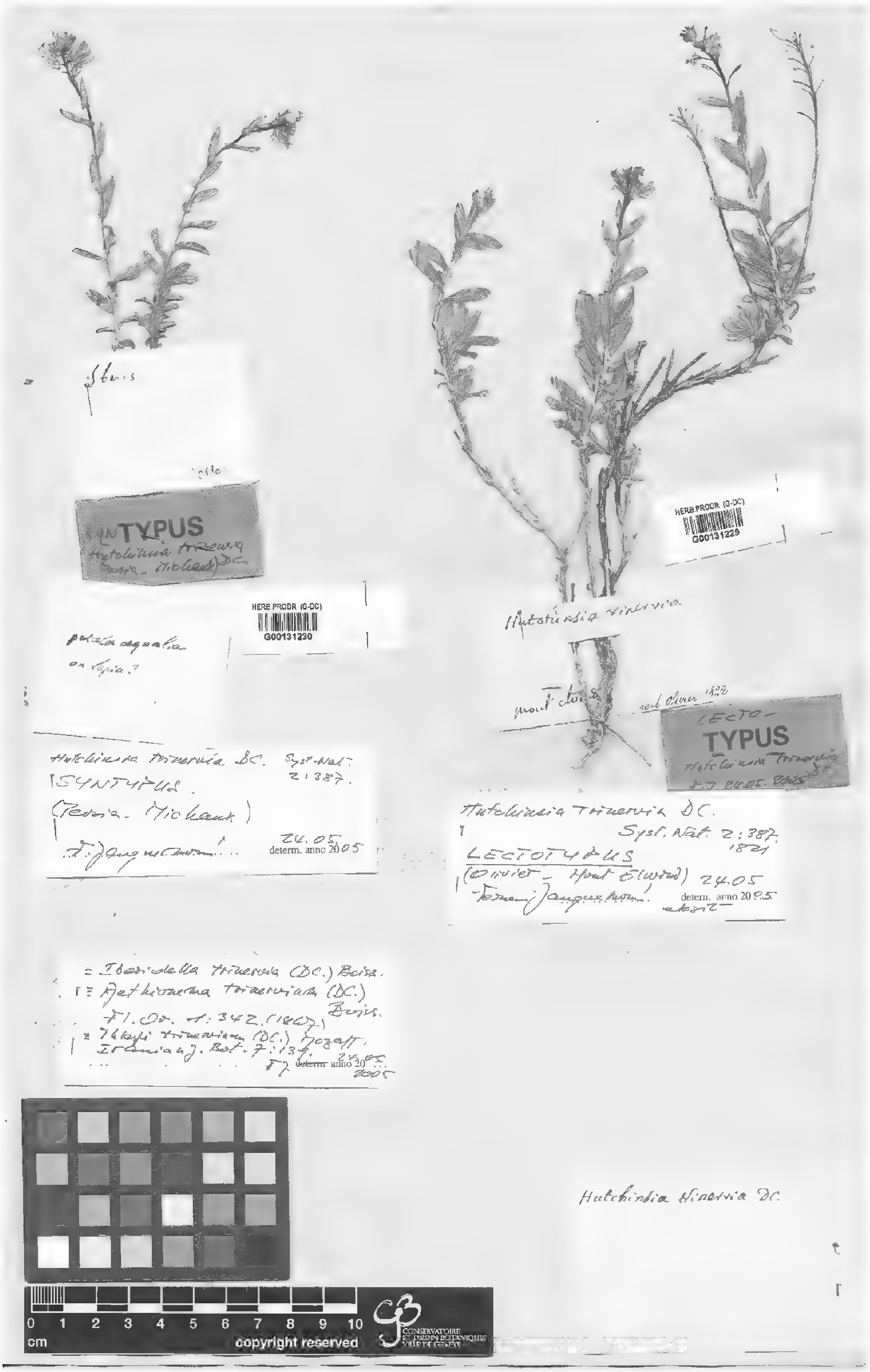

Figure 2. Lectotype specimen for *Vania trinervia* (DC.) Khosravi, Jacquemoud, Menke, Mumm. & Mohsenz. (*herb. Olivier 1822*, G-DC). Photo courtesy of Conservatoire botanique de la Ville de Genève.

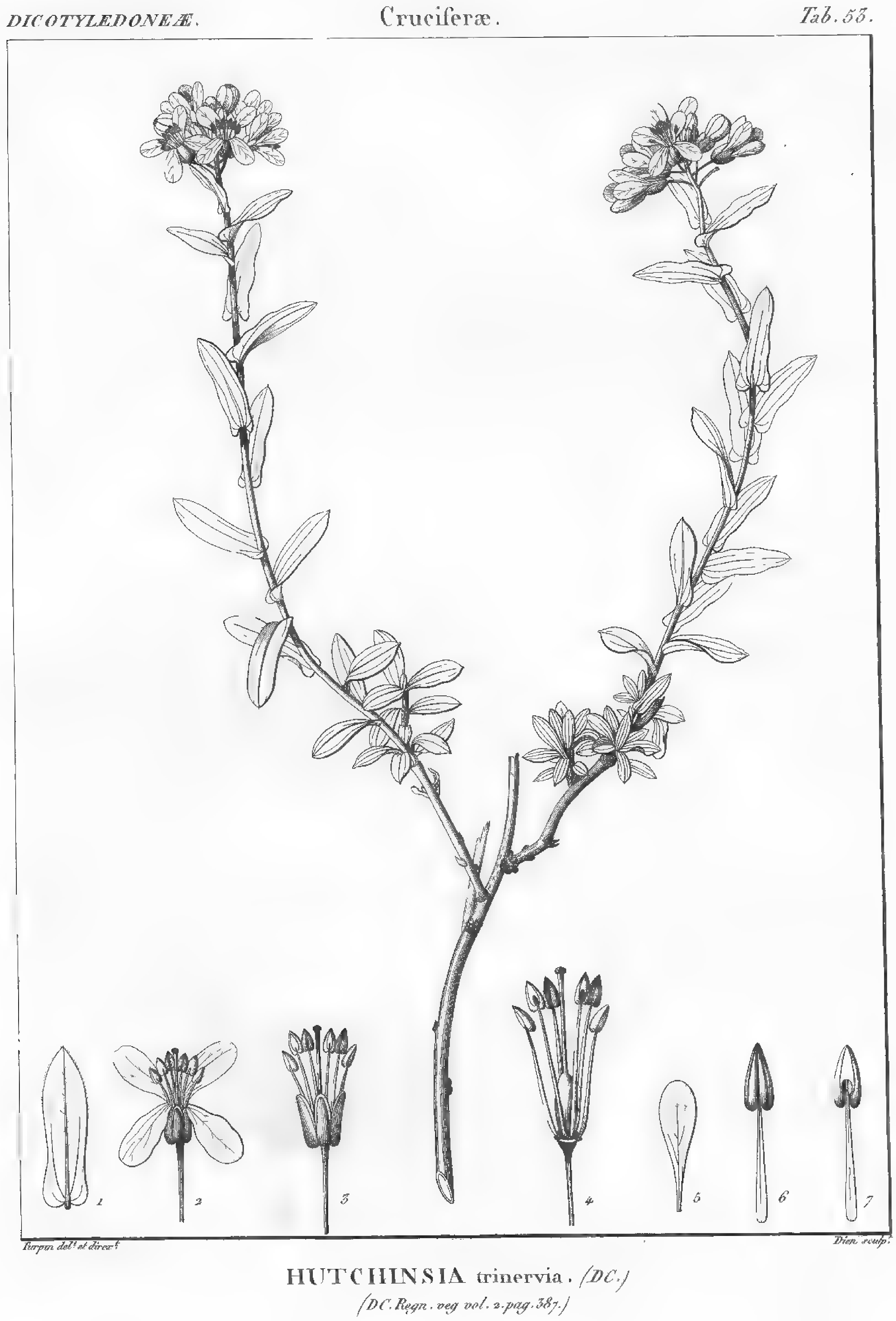

Figure 3. Engraving of the analytic illustration of *Hutchinsia trinervia* DC. by Turpin, in *Icones selectae plantarum* edited by Benjamin Delessert, with descriptions by A.-P. de Candolle. —1. Cauline leaf. —2. Flower. —3. Calyx with ovary and stamens. —4. Ovary and stamens. —5. Petal. —6. Stamen (internal view). —7. Stamen (external view). Courtesy of the Library, Conservatoire botanique de la Ville de Genève.

journey at Hamadan and collecting foray on "Mont Elvind" [Alwand Kuh]. However, neither Deleuze (1804: 198), the first biographer of Michaux, Sargent (1889: 3), or Boissier (1867: xxvi) provide any relevant information. More accurate data are found in Jaubert and Spach (1842–1843), since the book includes a map with the itineraries of the first botanical collectors in the Middle East. Particularly interesting are details of

Michaux's Persian itinerary, which were found in handwritten notes held by Delessert. It is unclear why no further information is available in Jaubert and Spach's discussion of Michaux. Furthermore, the absolute lack of reference to these notes by Lasègue (1845: 61), whose close familiarity with Delessert's herbarium, library, and archives is well known, is difficult to understand. Consequently, it is only known that Michaux traveled in Iran from 1783 to 1785 and went to Hamadan (according to the map in Jaubert & Spach [1842–1843]), certainly crossing part of the Alwand Kuh, likely on a similar route followed by Olivier and Bruguière about 10 years later in 1796–1797 (Boissier, 1867: xxvi). Finally, it should be noted that although Aucher-Eloy (1843) botanized twice on "Elwend" (25 and 29 May 1835) and provided a detailed report of his expedition, he does not refer to his compatriots Michaux, Olivier, or Bruguière, nor does he mention *Hutchinsia trinervia* (or *Aethionema trinervium*).

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